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Elemental analysis of chelant induced phytoextraction by *pteris vittata* using WD-XRF spectrometry

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Abstract

Soil pollution due to heavy metals derived from anthropogenic activities is one of the major global issues of our times. Detrimental effects of the heavy metals to the environment and human health are well understood now. Direct and multi-elemental quantitative analysis of soil and plant samples in chelant induced phytoaccumulation in *Pteris vittata* with the application of XRF spectrometry is the main aim of the present study. The chelant treatment of EDTA was effective for enhancing the arsenic (As) absorption in the pot experiments. Bioaccumulation factor for primary macronutrients P and K slightly decreased in roots but it increased considerably in fronds after the treatment. High increase in the bioaccumulation factor and translocation factor was recorded for As. At the end of this work, it can be clearly concluded that Wavelength Dispersive X-ray Fluorescence (WD-XRF) spectrometry can be successfully used in phytoremediation studies for getting good results in less time.

Highlights

- The chelant treatment of EDTA was effective for enhancing the arsenic bioabsorption in the pot experiments.
- WD-XRF spectrometry can be successfully used in phytoremediation studies for getting good results in less time.

Keywords: Bioaccumulation, chelating agents, heavy metals, phytoextraction, remediation, WD-XRF

Exposure of aquatic and terrestrial ecosystem to the excess amount of heavy metals has detrimental effects and is a major global issue prompting us to take concrete action. These heavy metals affect the soil, plant and human health to a large extent. Soils contaminated with high concentrations are often poor in nutrients, microbial diversity and phytotoxic to plants (Giller *et al*. 1998, Nagajyoti *et al*. 2010). Some of these metals are often required in trace amounts by the plants for their growth and development but at high concentrations cause phytotoxicity affecting overall health of plants (Kabata-Pendias 2010). Imposed risks of poisoning and toxicity to human and animal health are due to their inhalation, ingestion or absorption (Duruibe *et al*. 2007). These metals are found naturally in the soil but recent “progress” over the past few centuries by the mankind has added to the problem. Anthropogenic activities like industrial development and agricultural practices have disposed these metals to the soil aggregates (Wuana and Okieimen 2011). Biggest challenge to the environmentalists is to remove and reduce heavy metal contamination.

Different techniques are available to remediate the metal contaminated soil, *viz*. chemical, physical and biological techniques (McEldowney *et al*. 1993). Chemical and physical remediation is often costly. So, phytoextraction is one of the cost-effective techniques for enhanced remediation for metal contaminated soil. Phytoremediation can be defined as use of plants to remove, transfer and degrade contamination in soil, sediment or water (Hughes *et al*. 1997). This process includes a variety of remediation techniques which include many treatment strategies leading to contaminant degradation, removal (through accumulation or dissipation), or immobilization (Padmavathiamma and Li 2007).

P. vittata, also known as brake fern, is a perennial, evergreen fern native to China. It was first discovered arsenic hyperaccumulator as well as the first fern found to function as a hyperaccumulator (Ma *et al*. 2001). This fern possesses extraordinary ability for As hyperaccumulation (up to 22,600 mg As kg^{-1} in its fronds) (Ma *et al*. 2001), which is far greater than most plant species (<10 mg As kg^{-1}) (Matschullat 2000). Though at reduced rate *P. vittata* is effective in taking up As in the presence of other metals (Ni, Zn, Pb and Cd), but it had a limited capability to take up other metals (Fayiga *et al*. 2004). About a dozen of ferns belonging to genus *Pteris* are reported as As hyperaccumulator and few from others such as *Pityrogramma calomelanos* but not all members of the genus *Pteris* are able to hyperaccumulate arsenic (Xie *et al*. 2009). It was reported that plasma membranes of the root cells of *Pteris vittata* have a higher density of phosphate/ arsenate transporters than non-hyperaccumulator *P. tremula*, which may be as a result of constitutive gene overexpression (Caille *et al*. 2005). As hyperaccumulation by fern depends on high affinity to arsenate by the phosphate/ arsenate transport systems (Poynton *et al*. 2004) and the plant's capability to increase As bioavailability in the rhizosphere through reducing pH by root exudation of large amounts of dissolved organic carbon (Gonzaga *et al*. 2009). The decrease in pH increases water soluble As that can be readily taken up by the roots (Fitz and Wenzel 2002, Gonzaga *et al*. 2009).

Phytoextraction depends on the high biomass production capability and ability to accumulate large quantities of environmentally critical metals in the shoot tissue (Kumar *et al*. 1995, Blaylock *et al*. 1997, Prasad and Freitas 2003). Adding chelates or acidifying agents helps them to liberate into the soil solution, improving the metal accumulation capacities and uptake speed of non hyperaccumulating plants (Evangelou *et al*. 2007). Thus, when a metal does not exist in available form in the soil for sufficient plant uptake chelate-induced phytoextraction is considered. Over the past decades the use of persistent aminopolycarboxylic acids (APCAs) such as ethylene diamine tetraacetic acid (EDTA), biodegradable APCAs, ethylene diamine disuccinate (EDDS), nitrilo triacetic acid (NTA) and low molecular weight organic acids (LMWOA) have been used in various phytoextraction experiments (Evangelou *et al*. 2007). Limitation of addition of the chelating agents to the soil during the induced phytoextraction process is that it is toxic to the plants and has negative effect on the soil microbial health (Mühlbachová 2011). EDTA is a strong chelating agent, having strong complexes-forming ability (Yeh and Pan 2012).

In recent time, application of direct and multi-elemental quantitative analysis of soil and plant samples has been increased considerably. XRF spectrometry is one of such state-of-the-art techniques for elemental determination and quantisation in vegetal samples (Marguí and Hidalgo 2009). It provides excellent desired features, including multi-element capability, simple sample preparation, wide dynamic range, high throughput and low cost determination (Marguí and Hidalgo 2009). X-ray fluorescence analytical techniques find application in phytoremediation and plant biology studies (Nečemer *et al*. 2008).

The major aim of the present study was to know the elemental composition of *P. vittata* and to investigate the induced effect of EDTA on the bioaccumulation performance of the plant. Quantitative elemental analysis of dry soil and plant material was done using WDXRF, a new technique for geological and environmental materials.

Material and methods

Experimental design

The soil samples were collected from an area with naturally growing *P. vittata*, located at Ranibagh, Distt.-Nainital (Uttarakhand) India. Soil was air-dried and sieved through 5 mm sieve followed by filling in 5 kg plastic pots (25 cm diameter and 30 cm height). Two *P. vittata* plants of about 15 cm height were planted in each pot and no additives were added. The plants were allowed to grow under greenhouse conditions for 3 months. In the greenhouse the temperature of 28±2^{o}C with 12 hr day/night light cycle was maintained. To maintain moisture the pots were irrigated with tap water twice per week. After 3 months the soil in the pots was treated with chelating agent (EDTA) at the time of watering at a rate of 10 mM/kg soil. Control pots without any treatment of chelating agents were also included in the experiment. After adding chelating agent the plants were further allowed to grow for one more month. The pot experiment was carried out in triplicate. Thereafter the plants were harvested. The harvested plants were separated into two parts: aboveground (roots) and belowground parts (fronds) and washed thoroughly with deionised water 2-3 times to remove soil particles attached. Collected plant samples were oven-dried at 65°C to complete dryness and ground to fine powder using motor pestle, weighed and stored in air tight jars for further analysis.

WDXRF analysis

Samples were analysed using a commercial Wavelength Dispersive X-ray Fluorescence-S8 Tiger from Bruker (Germany), equipped with 4KWatt Rh anode X-ray tube with proportional flow counter and scintillation counter detectors. The instrument was capable of analyzing elements from carbon to uranium in the concentration range from PPM level to 100% in any form, i.e. liquid, solid or powder samples. Fine grounded samples of <100 mess size were taken for the analysis, which were then pelletized under 15 tons pressure using hydraulic press into pellets of 34 mm and a minimum thickness of ~ 3 mm. All the samples were analysed to record a whole spectrum for the identification of the elements in the samples. Quantitative analysis was done by the software provided with the instrument.

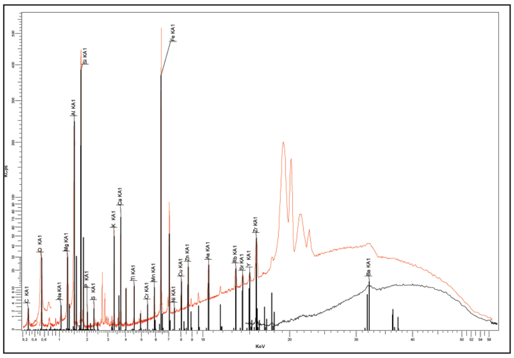
Results and discussion

The dry weight of the control and treated, roots and fronds did not show any consistent pattern (Table 1). WDXRF spectra of the soil; roots and fronds of the *Pteris vittata* are shown in figure 1. From the WDXRF multi-element spectral data concentrations of 22 elements (Si, Al, Fe, Ca, K, Mg, Na, Ti, P, S, Mn, Ba, Cl, Zr, Cr, Zn, Rb, Cu, Sr, Ni, As, Re) in experiment soil, roots and fronds (both control and treated) were determined (Table 2). In control, the roots recorded higher concentration of Si, Al, Fe, Ca, Mg, Na, Ti, Mn, Zr, Zn, Cr, Rb, Cu, Sr, Ni, As, "Re in roots" the fronds recorded higher concentrations of K, P, S, Ba, Cl. In treated experiments more concentration observed were those of Si, Al, Fe, Ca, Mg, Na, Ti, Mn, Ba, Zr, Cr, Zn, Cu, Sr, Ni, Re while K, P, S, Cl, Rb, As were observed in higher concentrations in the fronds. In roots of *P. vittata* increase in the accumulation was recorded for As, Cu, Zn, Re, Cr, S, Ca, Sr, Na, Mg and Al as compared to the control. Although increase in absorption of these 11 elements was observed but decrease was also observed in some elements. Reduced absorption was noted in Zr, Si, Mn, Ni, Rb, Cl, Fe, K and Ti. After the treatment there was no change in the Al, Fe and Na absorption in fronds of *P. vittata* as compared to the control (Table 2). In the treated fronds increased absorption was observed in As, K, P, Mg, Cu, Re, S, Cl, Ni, Cr and Rb while a drop in absorption was recorded for Ba, Si, Ti, Sr, Ca and Mn.

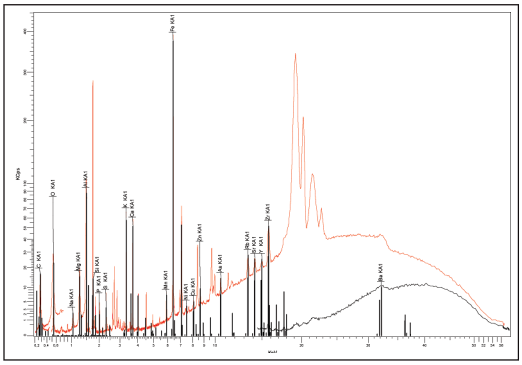
Table 1: Dry biomass yield of *Pteris vittata* grown in the control and treated soil

	Dry biomass of roots	Dry biomass of fronds	Total dry biomass
	(gm pot^{-1})	(gm pot^{-1})	(gm pot^{-1})
Control	5.0597±0.2731	12.3293±0.1412	17.3890±0.4079
Treated	5.1670±0.2409	12.1637±0.2497	17.3307±0.0904

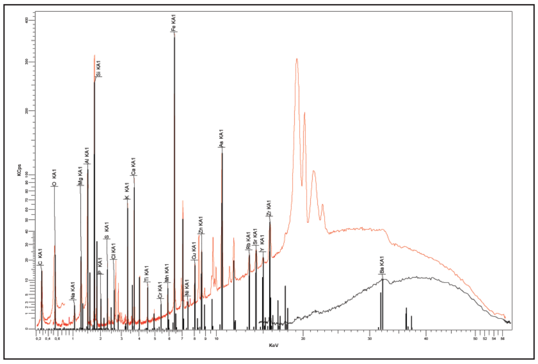
*Mean ± standard error



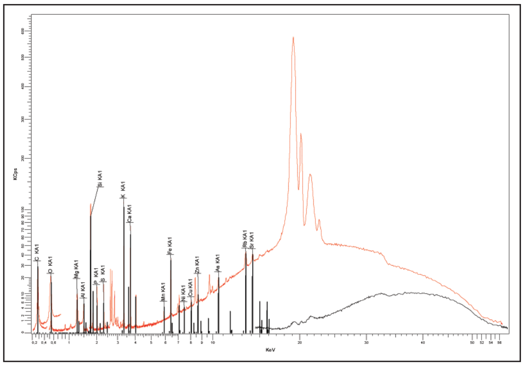
(a) Soil



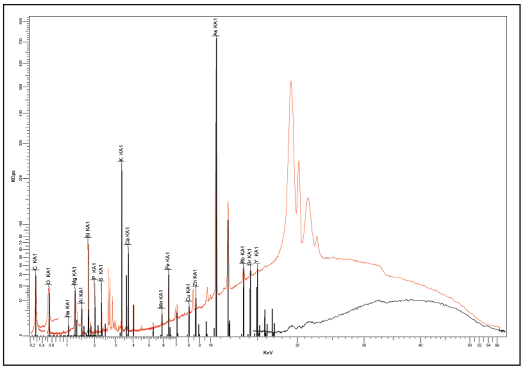
(b) Control roots



(c) Treated roots



(d) Control fronds



(e) Treated fronds

Fig. 1: WDXRF spectra of the soil; roots and fronds of the *Pteris vittata*

Table 2: Qualitative results of the WDXRF showing the concentration of different elements in soil; roots and fronds of the *Pteris vittata*

Elements	Soil	Control roots	Control fronds	Treated roots	Treated fronds
Si	245500	109000	37100	55500	23700
Al	61400	20300	900	20500	900
Fe	25300	10800	700	8700	700
Ca	26100	11100	10800	15000	10400
K	19000	13400	16800	11200	37200
Mg	16900	8300	4400	9200	6800
Na	4500	2400	500	3000	500
Ti	2900	1100	60	1000	56
P	2000	1600	3400	1400	5300
S	1200	1400	2000	2700	2900
Mn	700	300	100	200	99
Ba	400	-	83	100	-
Cl	300	2200	5400	1600	7300
Zr	200	200	-	100	-
Cr	300	50	15	100	16
Zn	400	1	-	3	1
Rb	80	48	35	33	36
Cu	300	27	16	200	24
Sr	35	28	17	34	16
Ni	26	21	6	14	7
As	200	38	26	700	3500
Re	-	200	68	600	100

*- below detectable limit, all the values are in mg kg^{-1}

To evaluate the ability of roots and fronds of *P. vittata* with respect to the element concentration in the soil, Bioaccumulation factor (BAF) was calculated separately for roots and fronds. BAF was calculated as follows: BAF$_{(r)}$= R$_{(c)}$/S$_{(c)}$ and BAF$_{(f)}$=F$_{(c)}$/S$_{(c)}$, where, BAF$_{(r)}$ is the bioaccumulation factor of roots, BAF$_{(f)}$ is the bioaccumulation factor of fronds, R$_{(c)}$ is concentration of element in the roots, F$_{(c)}$ is concentration of element in the fronds and S$_{(c)}$ is concentration of element in the soil.

Table 3: Bioaccumulation factor (BAF) of different elements in control and treated *Pteris vittata*

Elements	BAF$_{(r)}$ control	BAF$_{(r)}$ treated	BAF$_{(f)}$ control	BAF$_{(f)}$ treated
Si	0.444	0.226	0.151	0.097
Al	0.331	0.334	0.015	0.015
Fe	0.427	0.344	0.028	0.028
Ca	0.425	0.575	0.414	0.398
K	0.705	0.589	0.884	1.958
Mg	0.491	0.544	0.260	0.402
Na	0.533	0.667	0.111	0.111
Ti	0.379	0.345	0.021	0.019
P	0.800	0.700	1.700	2.650
S	1.167	2.250	1.667	2.417
Mn	0.429	0.286	0.143	0.141
Ba	0.000	0.250	0.208	0.000
Cl	7.333	5.333	18.000	24.333
Zr	1.000	0.500	0.000	0.000

Cr	0.167	0.333	0.050	0.053
Zn	0.003	0.008	0.000	0.003
Rb	0.600	0.413	0.438	0.450
Cu	0.090	0.667	0.053	0.080
Sr	0.800	0.971	0.486	0.457
Ni	0.808	0.538	0.231	0.269
As	0.190	3.500	0.130	17.500

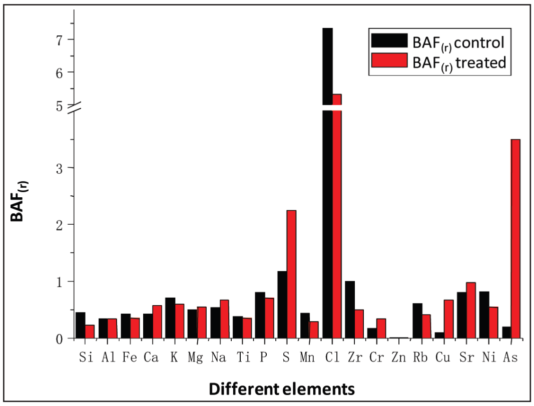


Fig. 2: Bioaccumulation factor (BAF) of different elements in roots of control and treated *Pteris vittata*

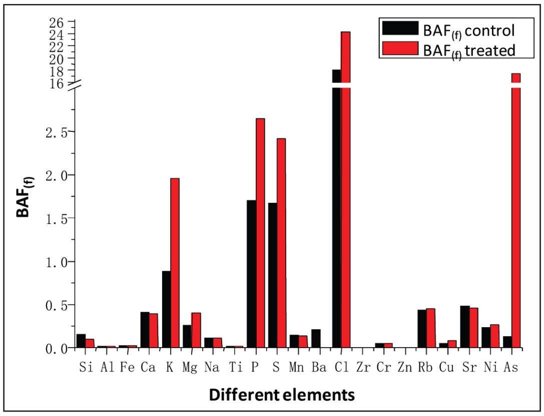


Fig. 3: Bioaccumulation factor (BAF) of different elements in fronds of control and treated *Pteris vittata*

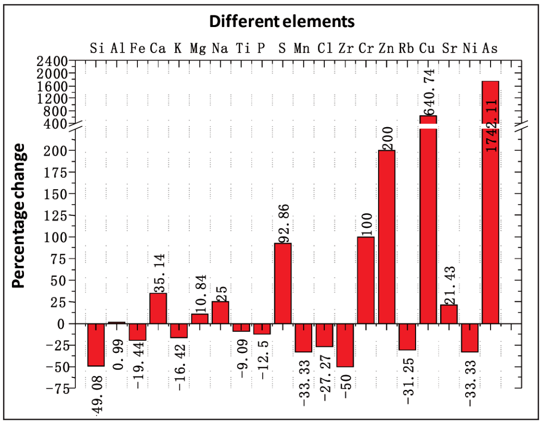


Fig. 4: Percentage change in the bioaccumulation factor (BAF) roots of different elements in *Pteris vitata* after treatment

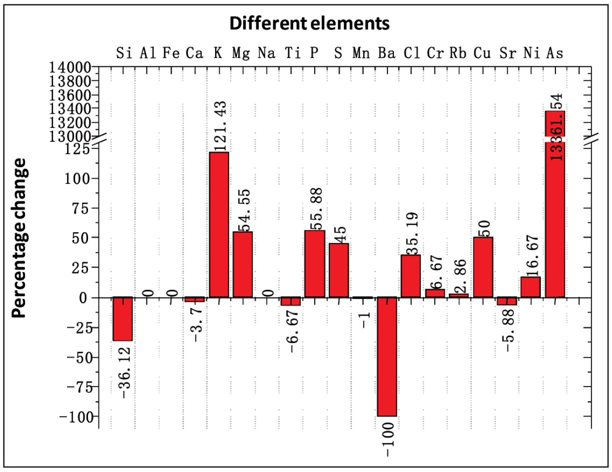


Fig. 5: Percentage change in the bioaccumulation factor (BAF) fronds of different elements in *Pteris vitata* after treatment

The result of the BAF for roots and fronds was presented in the Table 3. It was found that BAF was highest for the Cl for roots and fronds in both conditions, when no treatment was given and when treatment with chelating agent was done prior to harvesting. In the roots BAF was highest for Cl, 7.333 in control but after treatment it decreased to 5.333 while for As BAF was as low as 0.19 but it increased to 3.5 after treatment (Table 3, Figure 2). In fronds BAF was highest for Cl, 18 in control that after treatment increased to 24.333, followed by a BAF of 1.7 for P which increased to 2.65. In fronds the BAF for As was increased to 17.5 after treatment from 0.13 (Table 3, Figure 3). Percentage change in the BAF was calculated to estimate the increase or decrease after the treatment. It was observed that for roots after treatment BAF increase was highest for As (1742.11%) followed by Cu (640.74%), Zn (200%), Cr (100%), S (92.86%), Ca (35.14%), Na (25%), Sr (21.43%), Mg (10.84%), and Al (0.99%). It was found that decrease in BAF of roots was highest for Zr (50%) followed by Si (49.08%), Mn (33.33%), Ni (33.33%), Rb (31.25%), Cl (27.27%), Fe (19.44%), K (16.42%), P (12.5%) and Ti (9.09%) (Figure 4). No change was observed after treatment in the BAF of fronds for Al, Fe and Na while decrease was found highest for Ba (100%) followed by Si (36.12%), Ti (6.67%), Sr (5.88%), Ca (3.70%) and Mn (1%). Highest increase in BAF was observed for As (13361.54%) followed by K (121.43%), P (55.88%), Mg (54.55%), Cu (50%), S (45%), Cl (35.19%), Ni (16.67%), Cr (6.67%) and Rb (2.86%) after the treatment (Figure 5).

Translocation Factor (TF) or mobilization ratio of metals from roots to fronds has been estimated to determine relative translocation of elements from roots to fronds of *P. vittata*. TF was calculated as follows: TF= $F_{(c)}/R_{(c)}$ where $F_{(c)}$ is concentration of element in the fronds and $R_{(c)}$ is concentration of element in the roots.

Table 4: Translocation factor (TF) of different elements in control and treated *Pteris vitata*

Elements	TF control	TF treated
Si	0.340	0.427
Al	0.044	0.044
Fe	0.065	0.080
Ca	0.973	0.693
K	1.254	3.321
Mg	0.530	0.739
Na	0.208	0.167
Ti	0.055	0.056
P	2.125	3.786
S	1.429	1.074
Mn	0.333	0.495
Cl	2.455	4.563
Cr	0.300	0.160
Rb	0.729	1.091
Cu	0.593	0.120
Sr	0.607	0.471
Ni	0.286	0.500
As	0.684	5.000
Re	0.340	0.167

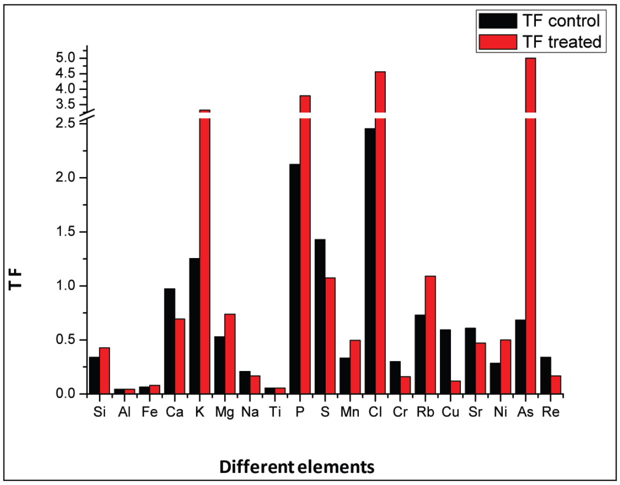


Fig. 6: Translocation factor (TF) of different elements in control and treated *Pteris vitata*

The result of the TF was presented in Table 4 and Figure 6. In the control the TF was highest for Cl (2.454) followed by P (2.125) and lowest for Al (0.044) while after treatment it recorded highest value for As (5) followed by Cl (4.562) and lowest for Al (0.043). Percentage change in the TF was calculated to estimate the increase or decrease after the treatment. It was found that after the treatment the TF increase was maximum for As (630.77%), followed by K (164.92%), Cl (85.88%), P (78.15%), Ni (75%), Rb (49.61%), Mn (48.5%), Mg (39.43%), Si (25.46%), Fe (24.14%) and Ti (2.67%) while decrease was maximum for Cu (79.75%), followed by Re (50.98%), Cr (46.67%), Ca (28.74%), S (24.81%), Sr (22.49%), Na (20%) and Al (0.98%) (Figure 7).

Various natural and synthetic enhancers are known to increase metal uptake. For example, sulphate and glutathione enhanced accumulation of arsenic in *Pteris vittata* (Wei *et al*. 2010). Enhanced Cu and Zn uptake by sunflowers were via citric acid addition (Yen and Pan 2012). In the present study although no significant different was observed in the dry biomass of roots and fronds (Table 1) but it was found that use of chelating agent before the harvesting has a significant effect on the absorption of different elements in the roots and fronds of *P. vittata* (Table 2). It was found that the concentration of the metal in the soil has the following trend: Si >Al>Ca>Fe>K>Mg>Na>Ti>P>S>Mn>Ba>Zn>Cl>Cr >Cu>Zr>As>Rb>Sr>Ni, while in the control roots it was Si>Al>K>Ca>Fe>Mg>Na>Cl>P>S>Ti>Mn>Zr >Re>Cr> Rb>As>Sr>Cu>Ni>Zn>Ba and in Control fronds it was Si>K>Ca>Cl>Mg>P>S> Al>Fe>Na>Mn>Ba>Re>Ti>Rb>As>Sr>Cu>Cr>Ni>Zr>Zn. After the treatment with the chelating agent we recorded the following trend: Si>Al>Ca>K>Mg>Fe>Na>S>Cl>P>Ti >As>Re>Mn>Cu>Ba>Cr>Zr>Sr>Rb>Ni>Zn for roots and K>Si>Ca>Cl>Mg>P> As>S>Al>Fe>Na>Re>Mn> Ti>Rb>Cu>Cr>Sr>Ni>Zn>Ba>Zr for fronds (Table 2).

Accumulation of selected elements varied greatly among different plant species and uptake of a particular element by a plant is primarily dependent on the plant species, its inherent controls and the soil quality (Chunilall *et al*. 2005). The presence of elements in the bioavailable form in the vicinity of the plant roots has a great impact on the bioabsorption of an element. When elements do not exist in available form in the soil for sufficient plant uptake, adding chelates or acidifying agents helps them to liberate into the soil solution, improving the metal accumulation capacities (Blaylock *et al*.

1997). Synthetic chelates such as EDTA have been shown to enhance phytoextraction of some heavy metals from polluted soil in previous studies (Grčman *et al*. 2001). In our study, we used EDTA as a chelating agent for treating the soil; it was found that increased accumulation was recorded for 11 elements compared to the control in roots of *P. vittata*. In fronds of *P. Vittata*, after the treatment, while there was no change in the Al, Fe and Na absorption and decrease in absorption of 6 elements was observed as compared to the control, but increased absorption was observed for 11 elements (Table 2, Figures 4 and 5). The degree of chelant induced extraction depends upon a number of factors like fractionation of metals retained in soil, types of chelating agents used and concentrations of chelating agents employed (Yeh and Pan 2012).

N, P and K are the primary macronutrients required by the plants for their growth and survival. We found that in the roots BAF of P and K was slightly decreased after the treatment but it increased considerably in fronds after the treatment (Figure 2 and 3). For secondary macronutrients S and Mg, after the treatment, BAF, both in roots and fronds, increased while for Ca it increased in roots and decreased slightly in fronds. This suggests non-significant effect on the health of the plant after treatment. Elements like B, Cu, Fe, Cl, Mn, Mo and Zn are also essential and are needed in only very small (micro) quantities, therefore called as micronutrients. Increase in the BAF was observed for Cu and Zn in roots and fronds, for Cl in fronds, while no change was observed for Fe in fronds (Figures 2 and 3).

Heavy metals like Pb, Cr, As, Zn, Cd, Cu, Hg, Al, and Ni when present in excess amount have well known associated environmental and health risks. In this study, Fe absorption decreased in the roots while it remained unchanged after the treatment. The absorption of Cr increased after the treatment in both above and underground part of the plant while it was more in the latter. Almost no change was observed in the absorption of Al, it increased slightly for Zn while increase was higher in the Cu in both the plant parts considered. Lou et al. (2007) found that chelating agents (EDTA, HEDTA) enhanced the Cu, Zn and Pb accumulation in three plant species including Chinese brake fern. But in our study elements like Pb, Cd and Hg were absent in both plant parts as they were also not present in the soil (Table 2). Other reason for this can be as result of the limited sensitivity of XRF instrumentation to Cd and Pb (Marguí and Hidalgo 2009).

Highest change was recorded for As for which this fern is well known. After the treatment the BAF for As increased by 1742.11% for roots and 13361.54% for fronds while TF increased by 630.77% (Figures 4, 5 and 7). Previously it was reported that EDTA and HEDTA lowered the As accumulation in this plant (Lou *et al*. 2007). This contrasting finding could be attributable to lower As level in the soil taken for the study as compared to theirs or may be due to chemical state in which it is present in the soil. It is believed that *P. vittata* has considerable ability to adjust As absorbing capacity under different soil As levels (Liao *et al*. 2004). In a previous study *P. vittata* was effective in taking up As (up to 4100 mg kg^{-1}) and transporting it to the fronds, but little in other metals (Fayiga *et al*. 2004). Our study revealed similar results. The BAF of fronds for As can be as low as 0.06-7.4 with a total 3-704 mg kg^{-1} As accumulation in the frond when soil As level was 51-261 mg kg^{-1} (Wei and Chen 2006). We recorded a BAF of 0.13 in the control while it was high (17.5) in the treated fronds. Ma *et al*. (2001) reported total 3-704 mg kg^{-1} As in plant after 6 weeks when 6-1500 mg kg^{-1} As was present in the soil. In the present study, after treating with chelating agent, we recorded 700 mg kg^{-1} As in roots and 3500 mg kg^{-1} As in fronds when 200 mg kg^{-1} As was present in the soil.

In addition to As hyperaccumulation from the soils this plant also enriched P and Ni from the soils and translocated them to the fronds (Liao *et al*. 2004). We found that after the treatment translocation factor for these two metals is further increased to about 78.15% for P and 75% for Ni (Figure 7). It was also found that *P. vittata* was also able to accumulate less known elements like Re, Sr, Rb, Zr, Ba and Ti. Use of chelating agents like EDTA, EDDS, citric acid and tartaric acid for the removal of heavy metals from contaminated soils by soil washing has been proposed by some researchers (Wuana *et al*. 2010) but the technique has its own limitation as it cannot be used on a large scale. Hence we suggest combining the use of chelating agents with the phytoaccumulating potential of plants for the enhanced remediation of contamination sites.

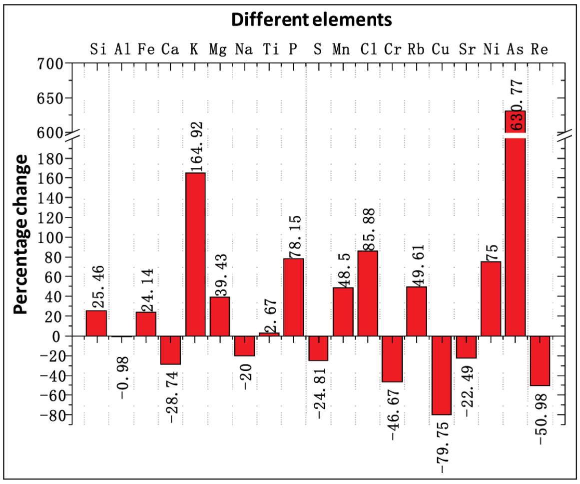


Fig. 7: Percentage change in the translocation factor (TF) of different elements in *Pteris vitata* after treatment

Conclusion and future prospective

Detrimental effects of heavy metals on the environment are well-revealing. *Pteris vittata* can be used as a valid tool for the effective remediation of the soil. The use of the chelant EDTA, was effective for enhancing the As absorption in the pot experiments. The application of chelant reorganized the bioaccumulation capability of *P. vittata*. Further study in relation to the use of chelating agents with the hyperaccumulator plants is needed to find practical feasibility at field level. Special care should be taken in the selection of suitable approach depending on the health attributes of the contamination site, target contaminant and efficacy of the plant selected. X-ray fluorescence-based techniques can be very useful for multi-element analysis, qualitatively and distribution in different plant parts with accuracy and reproducibility in less time. Future research should be focused on the combined use of more than one phytoremediation approaches for the successful remediation of the polluted area at the field conditions.

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